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In re Application of:	
FOSSHEIM et al.	Group Art Unit: 1615
Serial No.: 09/680,284	Examiner: Not assigned
Filed: October 6, 2000	

For: USE OF PARTICULATE CONTRAST AGENTS IN DIAGNOSTIC IMAGING FOR STUDYING PHYSIOLOGICAL PARAMETERS

COMPLETION OF CLAIM FOR PRIORITY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby submit the official certified copies of priority document numbers **GB 9807840.5** and **GB 9828874.9** in connection with the above identified application, benefit of which is claimed in the declaration of this application. The Examiner is most respectfully requested to acknowledge receipt of these certified copies in the next Official Action.

Respectfully submitted,

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December 21, 2000

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1.	**Your reference**	44.67555/000
2.	**Patent application number** *(The Patent Office will fill in this part)*	
3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Nycomed Imaging AS Nycoveien 1-2 N-0401 Oslo Norway
	Patents ADP number *(if you know it)*	06246961001
	If the applicant is a corporate body, give country/state of incorporation	Norway
4.	**Title of the invention**	Imaging
5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001

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Signature **Date** 9 April 1998
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Imaging

This invention relates to the use of particulate contrast agents in diagnostic imaging procedures for studying physiological parameters of the subject under investigation.

In diagnostic imaging procedures, e.g. X-ray, MRI, ultrasound, light imaging and nuclear imaging, it has long been known to use contrast agents to facilitate visualization of particular organs or tissues or to identify diseased or malfunctioning regions, ie. generating morphological images.

The present invention is concerned with the use of parenterally administered particulate contrast agents for the quantitative or qualitative study of physiological parameters within the human or non-animal (e.g. mammalian, avian or reptilian, but preferably mammalian) body.

Such parameters include for example pH, temperature, pressure, oxygen tension, carbon dioxide tension, the presence or concentration of other body metabolites or enzymes and cell surface properties, e.g. the presence or absence of various cell surface receptors. Parameters such as these may be indicative of the normal or abnormal functioning of the body as a whole or of a particular localized region, e.g. an organ which may or may not be tumorous, infected or otherwise malfunctioning. Likewise variations in such parameters may occur in response to drugs or other treatments administered to the body, e.g. hyperthermic treatment. As a result, quantitative, semi-quantitative or even qualitative determination of such parameters may be used to assess the need for a particular treatment or to monitor the success of a particular treatment.

pH and temperature are particularly important as indicators of abnormality or malfunction.

Several _in vivo_ methods, both imaging techniques and non-imaging techniques, can be used to study physiological parameters, e.g. to diagnose disease. Typical non-imaging techniques include simple blood pressure measurements, electrocardiography or electrocephalography for detection of electric currents in the heart muscle and brain, respectively, and other simple tests performed in doctors' offices or hospitals. Today, a variety of imaging techniques are also used. The most frequently used methods include various X-ray based techniques, MRI, ultrasound and diagnostic methods based on radioactive materials (e.g. scintigraphy, PET and SPECT). Other diagnostic imaging methods include light imaging modalities, Overhauser MR (OMRI), oxygen imaging (OXI) which is based on OMRI, magnetic source imaging (MSI), applied potential tomography (APT) and imaging methods based on microwaves.

The images obtained in X-ray techniques reflect the different densities of structures/organs/tissues in the patient's body. Contrast agents are today used to improve the image contrast in soft tissue examinations. Examples of such contrast agents include gas (negative contrast effect relative to tissue); barium sulphate suspensions; and iodinated agents including ionic monomeric agents, non-ionic monomers, ionic dimers and non-ionic dimers. Typical examples of commercial X-ray contrast agents are Omnipaque® and Visipaque®.

MRI is an imaging method generally based on interactions between radiowaves and body tissue water protons in a magnetic field. The contrast parameter or signal intensity is dependent on several factors including proton density, spin lattice (T_1) and spin spin (T_2) relaxation times of water protons. Typical commercial MRI contrast agents include Omniscan®, Magnevist® and ProHance®.

Ultrasound is another valuable modality in diagnostic imaging as it does not involve the use of

ionizing radiation. In ultrasound examinations the patient is generally exposed to sound waves in the frequency of 1-10 MHz. These sound waves (or ultrasound waves) penetrate through or are reflected from the tissue. The transmitted or reflected sound waves are detected by a "microphone" and form the basis for development of a ultrasound image. Ultrasound imaging is a method of choice in pregnancy checks and birth control and diagnosis of cardiovascular and liver diseases.

Although ultrasound contrast agents have been approved, there is as yet no general use of these agents. The main reason for this is the poor efficacy of the "first generation" agents. The ultrasound contrast agents currently under development are based on encapsulated gas because the reflection of sound from the liquid-gas interface is extremely efficient.

Typical ultrasound contrast agents are gas encapsulated in a sugar matrix, in a shell of denaturated albumin/or partly denaturated albumin, in polymers, and in surfactants including phospholipids. A typical ultrasound contrast agent with high contrast efficacy consists of a fluorinated gas bubble (for example SF_6 or a perfluorcarbon such as perfluoropropane or perfluorobutane) coated with a mono or multilayer phospholipid membrane. The particle size will generally be around 4 micrometer with very few particles larger than 10 micrometer in diameter. The main indications for such a typical product in the future may be cardiac imaging (cardiac perfusion examinations) and liver imaging.

Nuclear medicine imaging modalities are based upon administration of radioactive isotopes followed by detection of the isotopes, e.g. using gamma camera or positron emission tomography (PET). The most frequently used examination is gamma camera detection of 99-technetium in the form of a chelate, for example a

technetium phosphonate chelate for bone scintigraphy.

Light imaging methods are performed using contrast agents that absorb and/or emit light (generally near infrared light).

MSI methods may be performed without contrast agents; however, contrast agents based on magnetic materials improve this technique substantially.

APT based methods can also be performed (like for example thallium scans) without use of contrast agents; again however, contrast agents based on physiologically acceptable ions or other agents with effect on conductivity improve the diagnostic utility of APT.

All these different modalities complement each other with regard to diagnosis based on morphology/anatomy.

However, there has been a great interest in measurement and quantification of various physiological parameters. (See for example J. Magn. Reson. Imaging 1997, 7, 82-90 for a review on physiologic measurements by contrast enhanced MR imaging).

Various methods for measurements of physiologically important parameters have been described in the scientific literature: tissue pH has been measured using near infrared reflectance spectroscopy (J. Clin. Monit. 1996, 12, 387-95); intratumor pH has been measured using ^{19}F magnetic resonance spectroscopy (Invest. Radiol. 1996, 31, 680-9); 6-fluoropyridoxal polymer conjugates have been suggested as ^{19}F pH indicators for magnetic resonance spectroscopy (Bioconjug. Chem. 1996, 7, 536-40); spectral imaging microscopy has been used for simultaneous measurements of intracellular pH and Ca^{2+} in insulin-secreting cells (Am. J. Physiol. 1996, 270, 1438-46); fluorescence ratio imaging has been used for measurement of interstitial pH in solid tumors (Br. J. Cancer 1996, 74, 1206-15); a fluorinated pH probe for ^{19}F magnetic resonance spectroscopy has been used for in vivo pH measurement after hyperthermic treatment of

tumors in mice (Acta Radiol. 1996, 3, 5363-4); ^{31}P-NMR has been used for analysis of intracellular free magnesium and pH in erythrocytes (J. Soc. Gynecol. Investig. 1996, 3, 66-70); intracellular pH has been estimated in developing rodent embryos using computer imaging techniques (Teratology, 1995, 52, 160-8); biscarboxyethyl carboxyfluorescein has been evaluated as in vivo fluorescent pH indicator (J. Photochem. Photobiol. B. 1995, 227, 302-8); the effect of blood flow modification on intra- and extracellular pH has been measured by ^{31}P magnetic resonance spectroscopy in murine tumors (Br. J. Cancer, 1995, 72, 905-11); intracellular Ca^{2+}, pH and mitochondrial function in cultures of rabbit corneal tissue have been studied by digitized fluorescence imaging (In Vitro Cell Biol. Anim. 1995, 31, 499-507); a dual-emission fluorophore has been evaluated for fluorescence spectroscopy of pH in vivo (J. Photochem. Photobiol. B. 1995, 28, 19-23); nuclear magnetic resonance spectroscopy has been used to study lactate efflux and intracellular pH during hypoxia in rat cerebral cortex (Neurosci. Lett. 1994, 178, 111-4); ^{31}P NMR spectroscopy has been used for imaging of phosphoenergetic state and intracellular pH in human calf muscles after exercise (Magn. Reson. Imaging 1994, 12, 1121-6); multinuclear NMR spectroscopy has been used for studies of regulation of intracellular pH in neuronal and glial tumour cells (NMR Biomed. 1994, 7, 157-166), 5,6-carboxyfluorescein has been used as a pH sensitive fluorescent probe for in vivo pH measurement (Photochem. Photobiol. 1994, 60, 274-9); a fluorinated pH-probe has been used for non-invasive in vivo pH measurements (Invest. Radiol. 1994, 29, 220-2); fluorescence ratio imaging microscopy has been used for non-invasive measurement of interstitial pH profiles in normal and neoplastic tissue (Cancer Res. 1994, 54, 5670-4); 6-fluoro-pyridoxol has been used as probe of cellular pH using ^{19}F NMR spectroscopy (FEBS Lett. 1994,

349, 234-8); lactate and pH have been mapped in calf muscles of rats during ischemia/reperfusion assessed by in vivo proton and phosphorus magnetic resonance chemical shift imaging (Invest. Radiol. 1994, 29, 217-23); nuclear magnetic resonance spectroscopy has been used for measurement of in vivo and ex vivo pH (Eur. J. Lab. Med. 1996, 4, 143-156); seminaphthofluorescein-calcein has been tested as fluorescent probe for determination of intracellular pH by simultaneous dual-emission imaging laser scanning confocal microscopy (J. Cell Physiol. 1995, 164, 9-16); ampholytic dyes have been proposed for spectroscopic determination of pH in electrofocusing (J. Chromatogr. A 1995, 695, 113-122); EPR spectroscopy has been used for direct and continuous determination of pH values in nontransparent water-in-oil systems (Eur. J. Pharm. Sci. 1995, 3, 21-6); intracellular Ca^{2+} and pH have been imaged simultaneously in glomerular epithelial cells (Am. J. Physiol. Cell Physiol. 1993, 46, 216-230); fluorinated macromolecular probes have been evaluated for non-invasive assessment of pH by magnetic resonance spectroscopy (Bioorg. Med. Chem. Lett. 1993, 2, 187-192); pH has been mapped in living tissue by application of in vivo ^{31}P NMR chemical shift imaging (Magn. Res. Med. 1993, 29, 249-251); fluorescence spectroscopy has been used to measure temperature dependent aggregation of pH-sensitive phosphatidyl ethanolamine oleic acid-cholesterol liposomes (Anal. Biochem. 1992, 207, 109-113); ^{13}C NMR spectroscopy has been used to determine intracellular pH (Am. J. Physiol. Cell. Physiol. 1993, 264, C755-C760); ^{31}P NMR chemical shift imaging has been used for pH mapping of living tissue (Magn. Reson. Med. 1993, 29, 249-251); fluorescent probe and ^{31}P NMR spectroscopy have been compared for measurement of the intracellular pH of propionibacterium acnes (Can. J. Microbiol. 1993, 39, 180-6); panoramic imaging of brain pH and CBF has been performed during penicillin and metrazole induced status

epilepticus (Epilepsy Res. 1992, 13, 49-58); nuclear magnetic resonance spectroscopy has been used to study energy metabolism, intracellular pH and free Mg^{2+} concentration in the brain of transgenic mice (J. Neurochem. 1992, 58, 831-6); the pH dependence of 5-fluorouracil uptake has been observed by in vivo ^{31}P and ^{19}F nuclear magnetic spectroscopy (Cancer Res. 1991, 51, 5770-3); ^{31}P magnetic resonance spectroscopy has been used to study tumor pH and response to chemotherapy in non-Hodkin's lymphoma (Br. J. Radiol. 1991, 64, 923-8); ^{31}P magnetic resonance spectroscopy and microelectrodes have been used to evaluate dose-dependent thermal response of tumor pH and energy metabolism (Radiat. Res. 1991, 127, 177-183); hepatic intracellular pH has been studied in vivo by ^{19}F NMR spectroscopy (Magn. Reson. Med. 1991, 19, 386-392); the relationship between vertebral intraosseous pressure, pH, pO_2, pCO_2 and magnetic imaging signal inhomogeneity has been evaluated in a patient with back pain (Spine 1991, 16, 239-242); the effect of hypoxia on phosphorus metabolites and intracellular pH in the fetal rat brain have been studied by ^{31}P NMR spectroscopy (J. Physiol. 1990, 430, 98P); brain pH in head injury has been evaluated using image-guided ^{31}P magnetic resonance spectroscopy (Ann. Neurol. 1990, 28, 661-7); Se-labeled tertiary amines have been prepared and evaluated as brain pH imaging agents (Nucl. Med. Biol. Int. J. Radiat. Appl. Instrum. Part B 1990, 17, 601-7); ^{1}H, ^{31}P and ^{13}C nuclear magnetic resonance spectroscopy have been used to study cerebral energy metabolism and intracellular pH during severe hypoxia and recovery in the guinea pig cerebral cortex in vitro (J. Radiat. Appl. Instrum. Part B 1990, 26, 356-369); development of a pH-sensitive contrast agent for ^{1}H NMR imaging has been reported (Magn. Reson. Med. 1987, 5, 302-5); and there have been other references to ^{31}P NMR studies of pH, see for example Biomed. Res. (Japan) 1989 10, Suppl. 3, 587-597, J. Cereb. Blood Flow

Metab. 1990, 10, 221-6, Br. J. Radiol. 1990, 63, 120-4, Pediatr. Res. 1989, 25, 440-4, Radiology 1989, 170, 873-8, Cereb. Blood Flow Metab. 1988, 8, 816-821, J Neuro. Chem. 1988, U51U, 1501-9 abd Am. Heart J. 1988, 116 701-8.

One important physiological parameter of great medical interest has been temperature; temperature has been measured by electron paramagnetic resonance spectroscopy (J. Biomech. Eng. 1996, 118, 193-200), an ytterbium chelate has been used as a temperature sensitive probe for MR spectroscopy (Magn. Res. Med. 1996, 35, 648-651), fast imaging techniques have been evaluated in MRI for temperature imaging (J. Magn. Reson. B, 1996, 112, 86-90), ^{31}P and ^{1}H magnetic resonance spectroscopy has been used to study relationship between brain temperature and energy utilization rate in vivo (Pediatr. Res. 1995, 38, 919-925), local brain temperature has been estimated in vivo by ^{1}H NMR spectroscopy (J. Neurochem. 1995, 38,1995, 1224-30), magnetic resonance has been used to follow temperature changes during interstitial microwave heating (Med. Phys. 1997, 24, 269-277), the temperature dependence of canine brain tissue diffusion coefficient has been measured in vivo using magnetic resonance echoplanar imaging (Int. J. Hyperthermia 1995, 11, 73-86), temperature dependent ultrasound colour flow Doppler imaging has been carried out of experimental tumours in rabbits (Ultrasound Med. Biol. 1993, 19, 221-9), electrical impedance tomography has been proposed for temperature measurement (Trans ASME J. Biochem. Eng. 1996, 118, 193-200), temperature measurement has been carried out in vivo using a temperature-sensitive lanthanide complex and ^{1}H magnetic resonance spectroscopy (Magn. Res. Med. 1996, 35, 364-9), body temperature imaging by impedance CT has been carried out (Med. Imag. Tech. (Japan) 1995, 13, 696-702), temperature imaging has been carried out

inside the human body using microwaves (Med. Imag. Techn. (Japan) 1995, 13, 691-5), in vivo oxygen tension and temperature have been determined simultaneously using ^{19}F NMR spectroscopy of perfluorocarbon (Mag. Res. Med. 1993, 29, 296-302), measurement of in vivo pH in normal and tumor tissue has been carried out by localized spectroscopy using a fluorescent marker (Optical Eng. 1993, 32, 239-43), microwave temperature imaging has been proposed (IEEE Trans. Med. Imag. (USA) 1992, 4, 457-69), non-invasive temperature mapping during hyperthermia has been carried out by MR imaging of molecular diffusion (Proceedings of the Annual International Conference of the IEEE 1988, 342-343). There have been other reports of non-invasive and minimally invasive methods for the early detection of disease states by MRI, positron emission tomography, EEG imaging, MEG imaging, SPECT, electrical impedance tomography (APT), ECG imaging and optical diffusion tomography, see for example Proceedings of the SPIE - The International Society for Optical Engineering (USA) 1887 (1993).

Several patents and patent applications which relate to physiological imaging have been published: use of macrocyclic metal complexes as temperature probes for the determination of body temperature using spectroscopic methods with reduced background signals (WO94/27977); new fluorine containing macrocyclic metal complexes from tetraazadodecane derivatives useful for measuring tissue temperature from NMR chemical shift values, and as contrast agents for X-ray or NMR diagnosis (WO94/27978); determining and imaging of temperature change in human body using diffusion coefficients obtained by NMR to determine absolute temperature for individual points of body and temperature differences (WO90/02321); thermographic imaging using a temperature dependent paramagnetic material in an ESR enhanced magnetic resonance imaging

apparatus (WO90/02343); fluorosubstituted benzene derivatives useful as agents for in vivo NMR diagnosis, e.g. for measurement of tissue specific pH temperature, redox potentials, etc. (EP-A-368429); a magnetic resonance pulsed heat system for selectively heating a region of a subject that uses pulsed heat from focussed ultrasound equipment to destroy tumor tissue and MRI to provide fast scan images to monitor tissue and temperature with a diffusion sensitive pulse sequence (US-A-5247935); a magnetic resonance pulsed heat system for selectively heating tissue - surgery is performed using localised heating of tissue guided by and monitored by temperature sensitive magnetic resonance imaging and body tissue is heated using a magnetic resonance imaging system having a source and a probe containing a magnetic imaging coil and heating imaging rf source (US-A-5323778); apparatus for hyperthermia treatment of cancer comprising a combined hyperthermia and MRI probe to simultaneously heat a malignant area and monitor temperature, with a filter to isolate signals (WO91/07132); and a temperature measurement method using tomographic techniques of magnetic resonance imaging to measure the temperature of a region indirectly from an intensity change of magnetic resonance signal (US-A-5207222).

The present invention however is based on the understanding that particulate contrast agents may be produced in which the matrix or membrane material for the particles is responsive to a particular physiological parameter resulting in a change in the contrast efficacy of the contrast agent which may be correlated to that physiological parameter.

Thus viewed from one aspect the invention provides a method of imaging of an animate human or non-human animal body, which method comprises: administering parenterally to said body a particulate material comprising a matrix or membrane material and at least

one contrast generating species, which matrix or membrane material is responsive to a pre-selected physiological parameter whereby to alter the contrast efficacy of said species in response to a change in the value of said parameter; generating image data of at least part of said body in which said species is present; and generating therefrom a signal indicative of the value or variation of said parameter in said part of said body.

Viewed from a further aspect the invention provides a parenterally administrable contrast medium for imaging of a physiological parameter, said medium comprising a particulate material the particles whereof comprise a matrix or membrane material and at least one contrast generating species, said matrix or membrane material being responsive to said physiological parameter to cause the contrast efficacy of said contrast generating species to vary in response to said parameter. In a particularly preferred embodiment, the matrix or membrane material comprises a lipid or lipid mixture having a Tc value between 35 and 50°C, preferably between 37 and 45°C, more preferably between 38 and 43°C.

Viewed from a still further aspect the invention provides the use of a contrast generating species for the manufacture of a particulate contrast medium for use in a method of diagnosis comprising generating a signal indicative of the value of said physiological parameter, the particles of said contrast medium comprising a matrix or membrane material and at least one contrast generating species, said matrix or membrane material being responsive to said physiological parameter to cause the contrast efficacy of said contrast generating species to vary in response to said parameter.

In the method of the invention, the image data generated may if desired be presented as a two or more dimensional spatial image, alternatively they may be

presented as a temporal image, again in two or more dimensions. However in the extreme the data may simply provide one or more image values (e.g. numerical values) which either directly or indirectly may be used to provide quantitative or qualitative information (a signal) indicative of the value of the parameter under study. The image data may if desired be presented in visualizable form but alternatively they may simply be a set of data points which are collected and operated on to produce the signal without a visible image actually being generated. The signal indicative of the value of the parameter under study may likewise be generated in the form of a visible image, e.g. a map of the parameter value within the body, or a chart showing variation of the parameter value with time, or it may simply be a calculated numerical value for the parameter or an indication that the parameter is below or above a particular threshold value. Desirably, however, the signal provides a quantitative or at least semi-quantitative value for the parameter, e.g. either in a region of interest or in a plurality of regions of interest in the body, for example providing a spatial and/or temporal map of the parameter within at least a portion of the body.

The imaging technique used in the method of the invention may be any technique capable of use in conjunction with contrast agents, e.g. X-ray (e.g. CT scanning), MRI, MRS, MR microscopy, ESR imaging, ESR spectroscopy, Mössbauer imaging, ultrasound, light imaging, nuclear imaging (e.g. scintigraphy, PET or SPECT), MSI, APT, etc. In magnetic resonance techniques, signal strength or chemical shift or both may typically be studied. Preferably, the technique used will be an X-ray, MRI, ultrasound, light imaging or nuclear imaging technique, in particular an MRI or ultrasound technique. The particulate contrast agent used will accordingly be or contain a material capable

of having a contrast or signal generating effect in the particular imaging modality selected, e.g. a gas or gas precursor, a paramagnetic, ferromagnetic, ferrimagnetic or superparamagnetic material or a precursor therefor, hyperpolarized nmr active (ie. non zero nuclear spin) nuclei (e.g noble gas or ^{13}C nuclei), a radionuclide, a chromophore, (which term is used to include fluorescent and phosphorescent materials as well as light absorbers) or a precursor therefor, an ionic species, etc.

The physiological parameter studied using the method of the invention may be any physiochemical parameter capable of affecting the matrix or membrane material of the contrast agent, e.g. pressure, temperature, pH, oxygen tension, carbon dioxide tension, metabolite concentration, tissue electrical activity, tissue diffusion, etc. Preferably however it will be selected from blood parameters, e.g. pressure, temperature and pH, in particular in the vasculature rather than the chambers of the heart. It is not envisaged that the parameter be one which does not affect the membrane or matrix, for example flow rate or perfusion density.

A key part of the present invention is that the contrast agent particles should comprise a membrane or matrix material which is responsive to the physiological parameter under investigation so as to alter the contrast efficacy of the contrast agent. The manner in which the membrane or matrix responds will depend on the particular combination of imaging modality, physiological parameter and contrast generating material selected. Typically however the response might involve a change in membrane or matrix permeability to one or more species (e.g. water or gases), chemical or physical breakdown of the membrane or matrix material, generation of a contrast generating material, cleavage of functional groups from a contrast generating material thereby changing its contrast generating ability,

alteration of oxidation state in a contrast generating material thereby changing its contrast generating ability, etc. Such a response may thus for example involve release from the particulate contrast agent of water-soluble contrast generating moieties that are capable of being taken up into the extracellular fluid outside the vasculature. Particular examples of physiological parameter responsive particulate contrast agents will be described in greater detail below.

Thus one embodiment of the invention relates to thermosensitive paramagnetic particulate compositions for temperature MRI-mapping of the human body. Another embodiment of the invention relates to the use of thermosensitive particulate gas compositions as an ultrasound-based <u>in vivo</u> thermometer.

Yet another embodiment of the invention relates to radioactive compositions for temperature mapping in the human body. Another embodiment of the present invention relates to thermosensitive particulate compositions containing water-soluble X-ray contrast agents for mapping of temperature in the human body.

Still another aspect of the present invention relates to particulate compositions containing near infrared dyes for light imaging based temperature mapping in the body.

Another aspect of the present invention is to use one or more of the thermosensitive particulate compositions for temperature mapping in imaging guided hyperthermia treatment.

Another embodiment of the present invention relates to pH sensitive particulate compositions for determination of pH in the body. By way of example the active contrast agent (or indicator or probe) may be a paramagnetic, magnetic or fluorinated compound detectable by MRI. The active contrast agent (or indicator or probe) may be a gas or a gas generating substance for detection by ultrasound, it may be a

radioactive substance for detection by scintigraphy, SPECT or PET, or it may be a fluorescent dye, a near infrared dye, a UV dye or another dye that can be detected *in vivo* in light imaging or light detection methods.

Yet another embodiment of the invention relates to particulate compositions as contrast agents or as *in vivo* indicators or probes for detection of oxygen concentration/tension in the tissue using modalities such as ultrasound, MRI, Overhauser MRI and ESR.

Another embodiment of the present invention relates to particulate compositions as contrast agents or as *in vivo* indicators or probes for detecting pressure, turbulence, viscosity, enzyme activity, ion concentrations, metabolite diffusion coefficients, elasticity and flexibility.

Another aspect of the present invention relates to particulate compositions as contrast agents or as *in vivo* indicators or probes in combination with a targeting ligand, wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells, brain cells, and the glycoprotein GPIIb/IIIa receptor, for detection of changes in physiological parameters and/or quantification/ semiquantification of physiological parameters relevant for diagnosis of disease.

The particulate contrast agent may thus be used for quantification/semi-quantification of a physiological parameter which is relevant for diagnosis of disease. The particulate contrast agent may be triggered into giving a measurable signal difference either by the target parameter itself (e.g. the local temperature, pH or pressure or by binding to the particular cell surface receptors of interest) or by a chemical or biological response of the target parameter (e.g. release of enzymes or local variation in pH or temperature due to

cellular reactions). The particulate agent may thus respond to, identify and/or quantitatively or semi-quantitatively determine bacteria, viruses, antibodies, enzymes, drugs, toxins, etc.

Another aspect of the present invention relates to intravenous particulate compositions as contrast agents or as in vivo indicators or probes with long vascular half life (reduced liver uptake) for detection of changes in physiological parameters and/or quantification/semiquantification of physiological parameters relevant for diagnosis of disease.

The particulate contrast agent used according to the invention may be a solid material, a porous material, a liquid crystal material, a gel, a plastic material or a material having one or more walls or membranes. The chemical composition of the particulate material can be one simple chemical compound or a mixture of two or more chemical compounds. Generally it will comprise two or more different chemical entities, at least one of which is a matrix or membrane forming material and at least one other of which is a contrast generating species. The composition can consist of solid material(s) only or it may be a mixture of different solids/liquids/gases. The particulate will generally have a mean particle size (e.g. as determined by particle size analyzers such as laser light scattering apparatus or Coulter counters) in the range 0.001 to 20μm, more preferably 0.01 to 10 μm, especially 0.05 to 7 μm. Such particles are often described in the literature as particles, colloids, emulsions, droplets, microcrystals, nanocrystals, microparticles, nanoparticles, vesicles, liposomes, bubbles, microspheres, microbubbles, coated particles, microballons and the like.

The term "polymer" as used herein refers to any chemical compound with more than 10 repeating units. A polymer can be naturally occurring, synthetic, or

semisynthetic. Semisynthetic polymers are polymers that are produced by synthetic modification of naturally occurring polymers. Compounds with 2 to 10 repeating units are herein generally referred to as "oligomers" and likewise may be natural, synthetic or semisynthetic.

The term "surface active compound" or "surfactant" is used herein to refer to any chemical compound having at least one hydrophilic functional group and at least one hydrophobic (lipophilic) group. In a multiphase system, surface active compounds will commonly accumulate at the interface.

The term "lipid" is used herein to refer to naturally-occurring compounds, synthetic compounds and semisynthetic compounds which are surface active compounds and have structures similar to fatty acids, waxes, mono-, di- or tri-glycerides, glycolipids, phospholipids, higher (C_{10} or greater) aliphatic alcohols, terpenes and steroids.

The term "gas" is used herein to refer to any compound or a mixture of compounds with sufficiently high vapor pressure to be at least partly in the gas phase at 37°C.

When the imaging modality used according to the invention is ultrasound, the contrast generating species in the contrast agent will preferably consist of one or more encapsulated gases and/or one or more encapsulated gas precursors. This contrast generating species is able to interact with the surroundings so that the contrast agent gives information about one or more physiological parameters generally as a result of an interaction between the surroundings and the encapsulation material, if necessary followed by changes related to the gas/gas-precursor. However gaseous contrast generating species may be used in other imaging modalities, such as MRI and X-ray for example.

Typical examples of gas types that change contrast property as a result of the physiological parameters in

the surrounding tissue include: gases that are generated from a precursor as a result for example of pH, temperature or pressure changes, e.g. as a result of a chemical reaction, as a result of the boiling point of the gas, or as a result of a change of solubility; gases that compete with blood gases for absorption or adsorption sites within the matrix or membrane material; gases that change properties (e.g. lose hyperpolarization or change other magnetic properties) upon contact with body fluids or components, including dissolved components, thereof; gas molecules sensitive to pH; gases that change properties/volume with temperature; gases that change volume as a result of surrounding gas (e.g. oxygen tension); etc.

Preferred gases include hydrogen, oxygen, nitrogen, noble gases (including hyperpolarized gases), carbon dioxide, fluorinated gases (e.g. sulphur hexafluoride, fluorohydrocarbons, perfluorocarbons and other fluorinated halogenated organic compounds in gas phase), and low molecular weight hydrocarbons. Preferred gases also include any pharmaceutically acceptable gas mixture like for example air and air/perfluorocarbon mixtures. Preferably, the perfluorocarbon gas is selected from perfluoromethane, perfluoroethane, perfluoropropanes and perfluorobutanes. Any physiologically acceptable gas precursor can be used. Among suitable gas precursors are compounds that form a gas as a result of a chemical reaction (for example compounds sensitive to pH, for example carbonic acid, aminomalonic acid or other acceptable pH sensitive gas generating substances). Other suitable gas precursors are compounds that form a gas as a result of other physiological conditions like for example temperature, oxygen, enzymes or other physiological parameters/compounds relevant for body tissue (whether in the normal or diseased state) or which are activated to a gas forming state as a result of an interaction with an external stimulus (e.g. photo-

activation, sono-activation etc.).

The encapsulation material can be any material such as for example lipids, phospholipids, surfactants, proteins and polymers. Such materials may be chosen to dissolve, melt, collapse, weaken, increase porosity, or otherwise break down, change phase or change size (e.g. by aggregation due to change in surface charge, for example in response to local Ca^{2+} concentration) in response to the physiological parameter, e.g. to allow release of the contrast generating species into the surrounding fluid, or to allow body fluid or components thereof to come into contact with the contrast generating species, or to raise contrast agent species local concentration above the detection limit, etc. In this way the contrast generating effect of the contrast generating species may be dispersed (e.g. into the extracellular fluid space), switched on or increased (e.g. by generation of a contrast generating species such as a gas or by increasing water contact (for a positive (T_1 effect) MR contrast agent such as a gadolinium chelate)), or switched off or decreased (e.g. by destruction of the compartmentalization required for a negative (T_2 effect) MR contrast agent such as a dysprosium chelate, or by quenching of a radical or depolarization of a hyperpolarized nucleus or dissolution of a blood soluble gas). Moreover a porous solid matrix, e.g. a zeolite, may be impregnated with the contrast generating species with the pore mouths then being closed off totally or partially using a material which breaks down, melts or dissolves when the relevant physiological parameter (e.g. pH, temperature, enzyme concentration) in the surrounding body fluid is above or below a pre-set value.

The particulate contrast agent used according to the invention may respond to physiological parameters in several different ways. In one aspect, the particulate contrast agent may respond to physiological parameters

by accumulation in the area where a parameter is fulfilled, compared to areas where it is not. In another aspect of the invention, the particulate contrast agent responds by accumulation in areas where the physiological parameter is not fulfilled. In yet another aspect of the invention, the particulate contrast agent responds to a given parameter by disintegration, the disintegration being dissolution or chemical breakdown. Especially advantageous is a response to a physiological parameter by leakage or other transport means in/out of the particles. The opposite situation where the response to a physiological parameter is to prevent dissolution/leakage by attaining an increase in stability/reduction in membrane transport compared to particles in areas where the parameter is not fulfilled, is also a preferred aspect of the present invention. This type of response is advantageous since a time course may lead to a reduction in contrast by elimination from the organ in areas where the parameter is not fulfilled, while the contrast remains in the area of interest.

When a particulate composition responds by disintegration or transport, changes in contrast effect may be achieved by exposing otherwise invisible/shielded contrast agents, altering the distribution of contrast agents or, when the contrast agent is the particle itself (as in ultrasound contrast agents), destroying the contrast giving property. Especially advantageous are particulate compositions where the contrast effect is gained by interaction with the environment. In this case, both transport of the contrast agent and transport of the actual environmental component may be utilized for detection of physiological parameters. An example is MRI contrast agents where contact between the contrast agent and water leads to the measured contrast. In this case, response to a physiological parameter may be transport of water in/out of the particulate.

The leakage/transport of molecules in/out of a particulate may be accomplished in a variety of ways. All kinds of phase transitions may be utilized to induce leakage. For instance, a solid particle/ membrane may become leaky when it is melted, the process being sensitive to temperature. Phase transitions involving a gas phase may be used to respond to pressure as a physiological parameter. An especially useful aspect of the present invention is particles comprising liquid crystalline material as for example liposomes, niosomes or other vesicles. Liquid crystalline materials may undergo several different phase changes which may induce leakage or even breakdown of the particle. For example, the gel to liquid crystalline phase transition of phospholipids may induce leakage on heating and hence temperature sensitivity. The lamellar to reversed hexagonal phase transition will also induce leakage since the liposomes require lipids in lamellar, gel or other layered phase structure. The lamellar to reversed hexagonal phase transition may be induced by pH, electrolytes, and changes in the chemical environment such as targeting, enzymes, antibodies etc. The suitable parameter to respond to may be tuned by selection of the membrane composition and processing. Other phase transitions such as lamellar to cubic phases, lamellar to microemulsion phases or lamellar to normal hexagonal phase may also be used to introduce leakage.

Leakage may also be controlled by entities forming channels or other transport routes through the membrane of a particle. These channels may control the transport of molecules in/out of the particle, and be quite selective for, e.g., ions. For instance the protein tubulin which forms microtubules in absence of Ca^{2+} may induce a higher leakage in presence of Ca^{2+} than in absence of Ca^{2+} and hence be Ca^{2+} sensitive. Other proteins/enzymes which may control the transport of

substance in/out of a vesicle, include erythrocyte anion transporter, erythrocyte glucose transporter, Na^+-K^+ ATPase (Na^+/K^+ pump), Ca^{2+} - ATPase (Ca^{2+} pump) and Bacteriorhodopsin (H^+ - pump). Also biosurfactants such as iturins, esperine, bacillomycins, mycosubtilin, surfactin and similar substances may be used as membrane components to induce/prevent leakage by response to external parameters since these molecules may respond by changes in secondary and tertiary structure as well as self-assembly properties on influence from extrinsic parameters.

The contrast generating species in MR contrast agents used according to the invention will generally be a paramagnetic, superparamagnetic, ferrimagnetic or ferromagnetic compound and/or a compound containing other non zero spin nuclei than hydrogen, e.g. ^{19}F, ^{13}C, ^{15}N, ^{29}Si, ^{31}P and certain noble gases, such as ^{129}Xe or ^{3}He.

Preferred as paramagnetic compounds are stable free radicals, and compounds (especially chelates) of transition metal or lanthanide metals, e.g. manganese compounds, gadolinium chelates, ytterbium chelates and dysprosium chelates. Preferred magnetic (e.g. superparamagnetic) compounds are γ-Fe_2O_3, Fe_3O_4 and other iron/metal oxides with high magnetic susceptibility. Preferred fluorinated compounds are compounds with relative short ^{19}F T_1-relaxation times. Examples of MR contrast effective materials are well known from the patent literature, see for example the patent publications of Nycomed, Salutar, Sterling Winthrop, Schering, Squibb, Mallinckrodt, Guerbet and Bracco.

In general, there are two types of contrast generating species useful in MR contrast agents for use according to the invention: species that change contrast property as a result of the physiological parameters in the surrounding tissue; and species that are inert to physiology but change contrast properties as a result of

an interaction between coating material/encapsulation material and physiology. Typical examples here will be GdDTPA, GdDTPA-BMA, GdDOTA, GdHPDO3A in thermosensitive liposomes or in pH-sensitive vesicles.

Typical examples of species that change contrast property as a result of the physiological parameters in the surrounding tissue include: paramagnetic chelates that change relaxation properties and/or change chemical shift as a result of temperature, paramagnetic chelates that change coordination number and thereby relaxation properties and/or shift properties as a function of pH paramagnetic compounds, for example manganese compounds ($Mn(2+)/Mn(3+)$), europium compounds ($Eu(2+)$, $Eu(3+)$) and free radicals (radical, no radical) that change relaxation properties and/or shift properties as a result of oxygen tension/concentration or as a result of redox potential in the surrounding tissue, paramagnetic and magnetic compounds that change relaxation/shift properties as a result of enzymic activity (for example with enzymatic cleavage of paramagnetic chelates from macromolecules conjugated thereto causing a change in correlation time and/or water coordination) and paramagnetic chelates that change properties as a result of concentration of ions in the tissue, e.g. due to changes in water coordination.

The contrast generating species in X-ray contrast agents for use according to the invention will generally be a gas or gas generator or a water-soluble compound containing heavy atoms (e.g. atomic number of 37 or greater), e.g. metal chelates, metal clusters, metal cluster chelates and iodinated compounds. Preferred contrast generating species include ionic and non-ionic iodinated organic aromatic compounds, in particular triiodophenyl compounds. Most preferred are approved iodine based contrast agents such as salts, e.g. sodium or meglumine salts, of iodamide, iothalamate, diatrizoate, ioxaglate and metrizoate, and non-ionics

such as metrizamide (see DE-A-2031724), iopamidol (see BE-A-836355), iohexol (see GB-A-1548594), iotrolan (see EP-A-33426), iodecimol (see EP-A-49745), iodixanol (see EP-A-108638), ioglucol (see US-A-4314055), ioglucomide (see BE-A-846657), ioglunide (see DE-A-2456685), iogulamide (see BE-A-882309), iopromide (see DE-A-2909439), iosacol (see DE-A-3407473), iosimide (see DE-A-3001292), iotasul (see EP-A-22056), ioversol (see EP-A-83964) and ioxilan (see W087/00757).

Such contrast generating species may be incorporated into matrices or coatings that are sensitive to one or more physiological parameter.

The contrast generating species in nuclear medicine contrast agents for use according to the invention may be any radioactive compound of the type in diagnostic nuclear medicine, for example known compounds useful for scintigraphy, SPECT and PET. Typical compounds include radioiodinated compounds, ^{111}Indium labelled materials and ^{99m}Tc labelled compounds (for example ^{99m}TcDTPA, ^{99m}TcHIDA and ^{99m}Tc labelled polyphophonates) and ^{51}CrEDTA.

Such contrast generating species may be incorporated into matrices or coatings that are sensitive to one or more physiological parameter.

Contrast agents can be prepared for other imaging modalities such as light imaging, Overhauser MRI, oxygen imaging, magnetic source imaging and applied potential tomography, by encapsulation of the contrast generating species, e.g. a chromophore or fluorophore (preferably having an absorption or emission maximum in the range 600 to 1300nm, especially 700 to 1200nm), a stable free radical, a superparamagnetic particle or an ionic (preferably polyionic) species, for the respective modality into a physiologically sensitive matrix or coating.

<u>In vivo</u> temperature measurements have been of great interest because temperature is an important physiological parameter related to several indications

including cancer, cardiovascular diseases and inflammation. Local monitoring of temperature will also be of great value during hyperthermia treatment.

Contrast generating species can be released from the matrix/encapsulation material as a result of increased temperature and thereby change their contrast property or distribute to other tissues than the particulate product.

Typical examples of temperature sensitive particulate materials are temperature sensitive liposomes. These liposomes take the advantage of the fact that liposomes leak much more readily at the gel-to-liquid crystal phase transition temperature (Tc) of their membrane lipids. Liposomes made from specific phospholipids or a specific blend of phospholipids may be stable up to 37°C but break down or leak as they pass through an area of the body in which the temperature is raised, e.g. to 40 to 45°C, as a result of a disease process or an external heating. Table 1 below shows the transition temperature of various saturated phosphatidylcholines.

Table 1

Phosphatidylcholines (PC)	Transition temperature Tc (°C)
12:0	-1
13:0	14
14:0	23
15:0	33
16:0	41
17:0	48
18:0	55
19:0	60
20:0	66
21:0	72
22:0	75
23:0	79
24:0	80

Table 2 below shows the phase transition of various unsaturated phosphatidylcholines.

Table 2

Phosphatidylcholines (PC)	Transition temperature Tc (°C)
12:1	-36
18:1c9	-20
18:1t9	12
18:1c6	1
18:2	-53
18:3	60
18:4	-70

Table 3 below shows the phase transition temperature of various asymmetric phosphatidylcholines.

Table 3

Phosphatidylcholines (PC)	Transition temperature Tc (°C)
14:0-16:0	35
14:0-18:0	40
16:0-14:0	27
16:0-18:0	49
16:0-18:1	-2
16:0-22:6	-27
16:0-14:0	30
18:0-16:0	44
18:0-18:1	6
18:1-16:0	-9
18:1-18:0	9

Table 4 below shows the phase transition temperature for various saturated symmetric phosphatidylglycerols (PG) in the form of their sodium salts.

Table 4

Phosphatidylglycerols (PG)	Transition temperature Tc (°C)
12:0	-3
14:0	23
16:0	41
18:0	55

Tables 1-4 are based on information from the product catalogue of Avanti Polar Lipid Inc., USA.

Accordingly, phospholipids or blends of phospholipids may be selected to give products with the correct Tc for thermosensitive liposomes for diagnostic use. Typical blends for preparation of thermosensitive liposomes for diagnostic use are mixtures of dipalmitoylphosphatidylcholine (DPPC) and

dipalmitoylphosphatidyl glycerol (DPPG) and distearylphosphatidylcholine (DSPC).

Particulate contrast agents may also respond to temperature by utilizing the conformational temperature sensitivity of certain polymer systems. An example is poly(N-isopropyl acrylamide) which phase separates at 37°C. Hence particles comprising contrast agents will become leaky dependent on temperature (see Hoffmann et al. Macromol. Symp. 118: 553-563 (1997)).

Other examples of temperature sensitive matrices/coatings are lipid suspensions/emulsions containing the contrast generating species or other particulate or particulate like formulations that release the contrast generating species or change properties as a result of changes in temperature.

If the parameter under study is capable of manipulation, e.g. by treatment with drugs, external application of heat etc., it may be used to study the efficacy of such treatment or localized such treatment may be used to cause a change in contrast efficacy which in turn may be used to measure parameters such as organ perfusion. Thus for example external application of heat at, near or upstream of an organ of interest may be used to cause release from the particles of a contrast agent which may diffuse into the organ and so to detect blood perfusion (or lack of perfusion) in that organ. In this context one might administer a thermally sensitive particulate agent in connection with an external heating to follow the heat transport in parts of the body. Heat transport *in vivo* is directly connected to blood flow through the bioheat equation (J. Appl. Physiol. vol. 1, (1948), 93-122)

$$\frac{\delta T}{\delta t} r_t C_t + w_b c_b (T - T_a) = k\nabla^2 T + (Q_p + Q_m)$$

where r_t (kg/m^3) is the density of tissue, C_t ($J/kg°C$) is the specific heat of tissue, t (s) is the time, T (°C) is the temperature, w_b (kg/m^3s) is the blood perfusion, c_b (J/kg °C) is the specific heat of blood, T_a (°C) is the arterial temperature, k (W/m °C) is the thermal conductivity of tissue, Q_p (W/m^3) is the power deposition and Q_m (W/m^3) is the local metabolic rate. Hence, the thermosensitive particulate compositions may, after a controlled, localized external heating, give a measure of blood perfusion in an organ.

In vivo pH measurements have been of great interest because pH is an important physiological parameter associated to several severe diseases. The pH value is usually reduced during cancer diseases, cardiovascular diseases like for example stroke, osteoporosis, inflammations and autoimmune diseases.

One type of pH sensitive encapsulation for diagnostic agents involves the use of pH sensitive liposomes. The general strategy is to employ pH-sensitive groups in the liposomal membrane. Such typical groups have pKa values between 4 and 5.5. Phospholipids useful for preparation of pH-sensitive diagnostic agents include diheptadecanoyl phosphatidylcholine (DHPC) in admixture with DPPC and N-palmitoyl homocystein (PHC) in different ratios (see Eur. J. Pharm. Biopharm. 1993, 39, 97-101 for a general review on temperature and pH-sensitive liposomes).

Another type of pH-sensitive encapsulation of contrast generating species involves the use of pH-sensitive surfactants like for example N-dodecyl-2-imidazole propionate (DIP) which has pKa of 6.8 (see for example Pharm. Res. 1993, 13, 404). This means that DIP at pH 7.3-7.4 (physiological) is in the non-ionized (non/low surfactant activity) form (80%) while at for example lysosomal pH (5.2) over 97% will be in the charged form.

Another means of pH-sensitive encapsulation of

contrast generating species involves the use of matrix materials and/or coating materials with pKa values in the range of 4.5-7.0 so that the material is soluble or partly soluble in the charged form and insoluble or partly insoluble in the non-charged form. Such compounds can be physiologically acceptable low molecular weight compounds or physiologically acceptable polymers.

Still another means of pH-sensitive encapsulation involves the use of compounds that are chemically cleaved as a result of pH, for example polyorthoesters or polyacetals/ketals which are cleaved under acidic conditions.

Liposomes comprising phosphatidyl ethanolamines (PE) as the central component are another example of liposomes which can undergo a phase transition and become leaky when pH is reduced. pH sensitive liposomes can also be achieved by incorporation of fatty acids into phospholipid membranes.

In principle any charged particulate system where the charge is pH dependent and influences the packing of the membrane material can be used.

Access to oxygen is critical for all types of cells, and diagnostic agents for determination of oxygen concentration/tension in tissue will be of great importance in diagnosis of diseases like cancer, cardiovascular diseases, autoimmune diseases and several diseases in the central nervous system.

One type of oxygen or redox sensitive encapsulation/coating material is a material that has different solubility/diffusion properties dependent on the oxygen level or the redox status; for example compounds containing a nitro-group that is reduced *in vivo* to an amino-group which improves solubilization of the material in reductive/low oxygen surroundings.

Determination of concentration of physiologically important ions in tissue is important for several

diseases.

Types of ion concentration sensitive encapsulation materials that may be used in this regard include phospholipids, surfactants and other ion chelating materials. Negatively charged liposomes will for example bind Ca(2+) and the membrane will change its diffusion properties and become more stiff.

An example of Ca^{2+}/Mg^{2+} sensitive particulate compositions are liposomes enriched with the dimeric phospholipid cardiolipin. A cardiolipin containing membrane may undergo a lamellar to reversed hexagonal phase transition upon addition of the divalent cations since these ions bind to the cardiolipin di-phosphatidyl group.

Types of enzyme sensitive encapsulation material include matrices or coatings that are degraded by enzymes, for example simple esters of low molecular weight compounds or polyesters like polyacetic acid and others.

Various metabolites may also change the properties of coating materials.

Particulates can be made sensitive to for example antibodies based on enhanced leakaged due to a phase transition induced by the chemical binding between membrane molecules and the antibody. As an example, liposomes comprising N-(dinitrophenylamino-ε-caproyl)-phosphatidyl ethanolamine (DNP-cap-PE) become leaky due to a lamellar to reversed hexagonal phase transition when binding to anti-DNP. Another example includes liposomes comprising human glycophorin A in dioleoyl phosphatidyl ethanolamine membranes. These liposomes become leaky when immobilized antibodies are added.

A further aspect of the present invention is to use one of the above described particulate diagnostic agents together with another compound that has the potential to change the physiological parameter of interest or together with use of an external energy source to change

the parameter of interest.

Thus one example is to administer thermosensitive diagnostic agents in connection with an external heating and to follow the heating effect in parts of the body.

Another example is to administer compounds that change pH in connection with a pH-sensitive particulate diagnostic agent to follow the pH-profile in the area of interest

Still another example is to cause the subject under study to inhale oxygen, after administration of an oxygen sensitive diagnostic agent, to follow oxygen uptake in tissue.

Early diagnosis is very important to obtain good therapeutic results. In most disease processes changes in physiological parameters take place before changes in morphology. All existing contrast agents diagnose morphology. The new types of contrast agent according to the invention are able to detect diseases at a very early stage in the disease process and thereby improve the therapeutic outcome for the patient.

Where the particulate diagnostic agent or a component thereof carries an overall charge, it will conveniently be used in the form of a salt with a physiologically acceptable counterion, for example an ammonium, substituted ammonium, alkali metal or alkaline earth metal cation or an anion deriving from an inorganic or organic acid. In this regard, meglumine salts are particularly preferred.

The diagnostic agents of the present invention may be formulated in conventional pharmaceutical or veterinary parenteral administration forms, e.g. suspensions, dispersions, etc., for example in an aqueous vehicle such as water for injections.

Such compositions may further contain pharmaceutically acceptable diluents and excipients and formulation aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH

adjusting agents, etc.

Where the agent is formulated in a ready-to-use form for parenteral administration, the carrier medium is preferably isotonic or somewhat hypertonic.

Where the particulate agent comprises a chelate or salt of an otherwise toxic metal species, e.g. a heavy metal ion, it may be desirable to include within the formulation a slight excess of a chelating agent, e.g. as discussed by Schering in DE-A-3640708, or more preferably a slight excess of the calcium salt of such a chelating agent.

The dosage of the diagnostic agents of the invention will depend upon the imaging modality, the contrast generating species and the means by which contrast enhancement occurs (e.g. with switching on or off of contrast, with dispersion of contrast out of the vascular space, etc).

In general however dosages will be between 1/10 and 10 times the dosage conventionally used for the selected contrast generating species or analogous species in the same imaging modality. Even lower doses may also be used.

While the present invention is particularly suitable for methods involving parenteral administration of the particulate material, e.g. into the vasculature or directly into an organ or muscle tissue, it is also applicable where administration is not via a parenteral route, e.g. where administration is transdermal, nasal, sub-lingual or is into an externally voding body cavity, e.g. the gi tract, the bladder, the uterus or the vagina. The present invention is deemed to extend to cover such administration.

The disclosures of all the documents mentioned herein are incorporated by reference.

The present invention will now be illustrated further by reference to the following non-limiting Examples.

Example 1

Preparation of Temperature Sensitive Paramagnetic Liposomes

Liposomes containing GdHPDO3A (ProHance®, Bracco Spa, Milan, Italy) and GdDTPA-BMA (Omniscan®, Nycomed Amersham Imaging AS, Oslo, Norway) were prepared by the thin film hydration method. Two different saturated phospholipid blends were used; one consisting of hydrogenated phosphatidyl choline (HPC) (Lipoid GmbH, Ludwigshafen, German) and hydrogenated phosphatidylserine-sodium (HPS) (NOF Corporation, Amagasaki, Japan); the other composed of DPPC and DPPG-sodium (Sygena Ltd, Liestal, Switzerland). The phospholipid mixtures contained 5% or 10% (w/w) of the negatively charged HPS and DPPG components. The total lipid concentration was 50 mg/ml. The liposomes were subjected to 3 freeze-thaw cycles in liquid nitrogen. Differently sized liposomes were produced by sequential extrusion (Lipex Extruder®, Lipex Biomembranes Inc., Vancouver, Canada) through polycarbonate filters of various pore diameters. Untrapped metal chelate was removed by gel filtration or dialysis.

Physiochemical Properties

The mean hydrodynamic diameter of the liposomes varied from 103 nm to 276 nm, as measured by photon correlation spectroscopy (ZetaSizer IV, Malvern Instruments Ltd., Malvern, England); the zeta potential was negative in the order of -25 mV, as determined by laser Doppler velocimetry at 25°C (ZetaSizer IV, Malvern Instruments Ltd., Malvern, England). The mean gel-to-liquid crystalline phase transition temperature (Tc) of the HPC/HPS and DPPC/DPPG preparations was 50 and 42°C, respectively, as determined by differential scanning calorimetry (DSC4, Perkin Elmer Inc., Norwalk, CT).

Temperature

Figure 1 of the accompanying drawings and Table 5 below show the temperature sensitivity of in vitro r_1 relaxivity for liposome encapsulated GdDTPA-BMA and GdHPDO3A, respectively. Figure 2 of the accompanying drawings shows the temperature response of the in vitro MR signal intensity for liposome encapsulated GdDTPA-BMA.

Table 5

Temperature (°C)	r_1 (s^{-1} mM^{-1})		
	DPPC/DPPG 103 nm	HPC/HPS 130 nm	Control*
20	0.16	0.06	4.53
25	0.23	0.08	4.27
30	0.31	0.12	3.94
37	0.69	0.21	3.75
45	3.30	0.53	3.07
55	3.10	3.00	2.82
60	-	2.96	2.54

*non-liposomal GdHPDO3A

Claims

1. A method of imaging of an animate human or non-human animal body, which method comprises: administering parenterally to said body a particulate material comprising a matrix or membrane material and at least one contrast generating species, which matrix or membrane material is responsive to a pre-selected physiological parameter whereby to alter the contrast efficacy of said species in response to a change in the value of said parameter; generating image data of at least part of said body in which said species is present; and generating therefrom a signal indicative of the value or variation of said parameter in said part of said body.

2. A contrast medium for imaging of a physiological parameter, said medium comprising a particulate material the particles whereof comprise a matrix or membrane material and at least one contrast generating species, said matrix or membrane material being responsive to said physiological parameter to cause the contrast efficacy of said contrast generating species to vary in response to said parameter.

3. The use of a contrast generating species for the manufacture of a particulate contrast medium for use in a method of diagnosis comprising generating a signal indicative of the value of said physiological parameter, the particles of said contrast medium comprising a matrix or membrane material and at least one contrast generating species, said matrix or membrane material being responsive to said physiological parameter to cause the contrast efficacy of said contrast generating species to vary in response to said parameter.

1/2

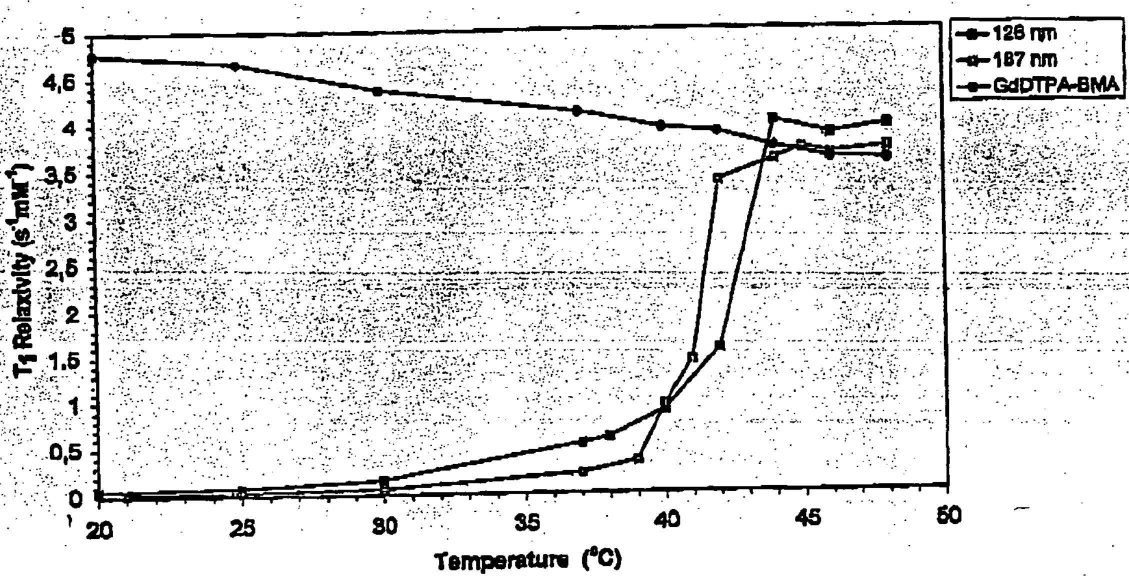

Figure 1. Temperature response of <u>in vitro</u> r_1 for GdDTPA-BMA encapsulated in DPPC/DPPG liposomes (0.47 T)

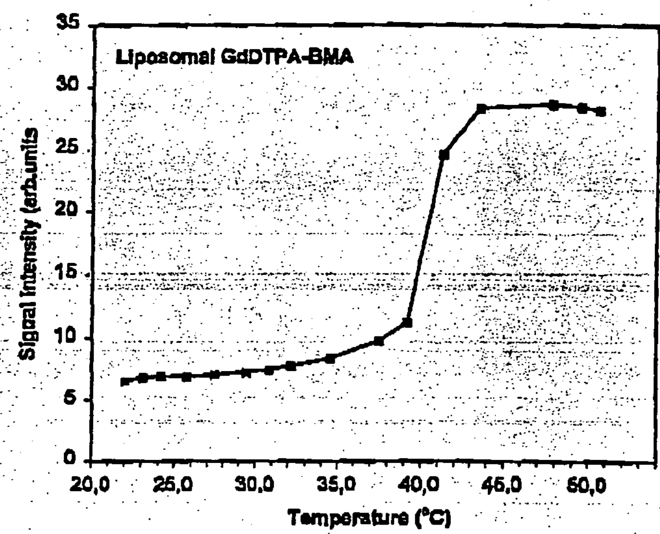

Figure 2. Temperature response of MR signal intensity for GdDTPA-BMA encapsulated within DPPC/DPPG liposomes (2.0 T).